Biochimica et Biophysica Acta, 514 (1978) 54—68

BBA 78199

ESTIMATION OF MOLECULAR AVERAGES AND EQUILIBRIUM FLUCTUATIONS IN LIPID BILAYER SYSTEMS FROM THE EXCESS HEAT CAPACITY FUNCTION

ERNESTO FREIRE and RODNEY BILTONEN

Departments of Biochemistry and Pharmacology, University of Virginia School of Medicine, Charlottesville, Va. 22903 (U.S.A.)

(Received March 28th, 1978)
(Revised manuscript received June 27th, 1978)

Summary

It is demonstrated that the bilayer partition function can be numerically obtained from scanning calorimetric data without assuming a particular model for the gel-liquid crystalline transition. From this partition function, the enthalpy, entropy and volume changes accompanying the transition can be calculated. In the limit of very large systems, the method of the grand partition function allows calculation of cluster model distribution functions from which average sizes of gel and liquid-crystal clusters, cluster densities and equilibrium fluctuations are obtained. These results indicate that the main transition in phospholipid bilayers proceeds through the formation of clusters and that these clusters are not static domains but highly fluctuating entities. These fluctuations in cluster size are approximately equal to the average cluster size and give rise to localized density and volume fluctuations. The magnitude of these fluctuations is affected by the radius of curvature of the bilayer and by the addition of small molecular weight compounds to the system.

Introduction

The gel-liquid crystalline transition of phospholipid bilayer systems has been the object of a great deal of experimental and theoretical work. This work has shown that the thermodynamic characteristics of the transition are greatly influenced by the phospholipid chain length [1], the radius of curvature of the bilayer [2] and by the addition of small molecular weight compounds such as anesthetics [3] to the system. Several workers [4—8] have considered various

Abbreviation: ANS, 8-anilino-1-napthalene sulfonate.

statistical mechanical models to describe this transition in phospholipid bilayers. However, this work has been primarily directed toward formulating plausible expressions for the possible configurational states of the hydrocarbon chains of the phospholipids and not for the configurational states of the bilayer as a whole.

Although it is recognized that the properties of the bilayer have their ultimate origin in the configurational state of the phospholipid molecules, there does not appear to be a simple one-to-one correspondence between the properties of the bilayer and their isolated constituents. For example, there is strong experimental evidence which indicates that transport properties across the membrane do not directly depend on the degree of fluidity of the phospholipids. Marsh et al. [9] reported that the permeability of the bilayer to Tempocholine has a sharp maximum at the lipid phase transition temperature. Similarly, Tsong et al. [10] have shown that the rate of permeation of 8-anilino-1-napthalenesulfonate (ANS) into DML vesicles is maximal at the transition temperature and that this rate is affected by the addition of local anesthetics to the system. It was also recently reported [11] that incorporation of M13 virus coat protein into synthetic lecithin vesicles has a sharp maximum at the transition temperature. These and similar phenomena do not appear to be simply correlated to the degree of fluidity of the bilayer or the average conformational state of the phospholipids, but rather to the particular way in which the phospholipid molecules are organized and distributed within the bilayer. In this respect, it has been suggested [3,9,10] that gel and liquid crystal clusters may actually form and coexist in dynamic equilibrium during the transition. However, the possibilities of an exact theoretical treatment of this system are limited by the fact that its partition function cannot be solved exactly.

Recently, Freire and Biltonen [12,14] have developed an alternative treatment based upon the result that the partition function of a system can be experimentally obtained from scanning calorimetric data without a priori assuming a model for the transition. The same authors have also shown using the cluster model [13,14] that, in the limit of very large systems, cluster averages and cluster distribution functions can be calculated from experimental partition functions. In this communication, the deconvolution theory of cooperative transitions is applied to the case of phospholipid bilayers. Heat capacity data for the main transition of multilamellar liposomes prepared from dipalmitoyl phosphatidylcholine in the absence and in the presence of the gaseous anesthetic halothane and sonicated single-lamellar dipalmitoyl phosphatidylcholine vesicles are analyzed within the context of this theory.

Theory

Specification of the cluster model for the system. During the gel-liquid crystalline transition each phospholipid molecule which melts can either create a liquid cluster of unit size, increase the size of a pre-existing cluster by one or join two (or more) already existing clusters. Accordingly, the phospholipid bilayer can be thought of as being formed of clusters of phospholipids in the gel state and clusters of phospholipids in the liquid crystal state. At

temperatures below the transition temperature, T_m, all phospholipids are in the gel state and the entire bilayer can be considered as being a single gel cluster. In the transition region, where the number of phospholipid molecules in the gel and liquid crystal states is finite, clusters of phospholipids in the gel state and clusters of phospolipids in the liquid crystal state will be formed and coexist within the bilayer. The size and number of these clusters will depend on the fractional degree of melting and the magnitude of the cooperative interactions existing within the bilayer.

Cooperativity, within this context, is defined as the tendency of the residues in the same state to group together. The transition would be infinitely cooperative if only two states were available to the bilayer: all lipid molecules in the gel state, or all lipid molecules in the liquid crystal state.

The actual nature of the transition (which lies somewhere between the non-cooperative and infinitely cooperative cases) will depend on the ultimate balance between two forces: the combinatorial entropy which tends to maximize the number of clusters, minimizing their size, and the cooperative forces which tend to maximize the cluster size, minimizing their number. The first one has its origin in the combinatorial degeneracy and is entropic in nature. This combinatorial degeneracy arises from the many different ways of distributing a given number of gel and liquid phospholipid molecules within the bilayer lattice. The cooperative forces, on the other hand, arise from different kinds of interactions whose molecular origins are still not understood. In any case, cooperative interactions cause gel-gel or liquid-liquid neighbors to be energetically more favorable than gel-liquid neighbors.

In this representation of the bilayer, the gel-liquid crystalline transition is viewed both in terms of the melting of the individual phospholipid molecules, and in terms of cluster formation and disruption. This view of the transition is similar to that espoused by Marsh et al. [9], Tsong et al. [10] and Lee [15]. At any temperature the configurational state of the bilayer can be described in terms of the fractional number of phospholipids in the gel and liquid states, the average size and number of liquid crystal and gel clusters, and fluctuations of these quantities about their equilibrium values.

These thermodynamic averages can be expressed in terms of the bilayer partition function, $Z(N)$, which is obtained by summing the statistical weights, W_c, of all the possible configurations, c, available to a bilayer of N phospholipid molecules,

$$Z(N) = \sum_c W_c \tag{1}$$

Eqn. 1 cannot be solved exactly and, thus, an analytical expression for the bilayer partition function is currently unavailable. However, this fact does not preclude a statistical thermodynamic analysis of the transition since it has been demonstrated [12] that the partition function of a system can be numerically evaluated from scanning calorimetric data and that this procedure is equivalent to evaluating the sum in Eqn. 1.

Experimental determination of the bilayer partition function. Before we proceed, it is useful to define reference states to which to refer the relative statistical weights and the excess thermodynamic quantities associated with the

transition. Thus, we will introduce the partition functions $Q(N)$ and $Z(N)$ defined as follows. $Q(N)$ is the ratio between $Z(N)$ and the statistical weight of the configuration in which all phospholipids exist in the gel state. $Z(N)$ is the ratio between $Z(N)$ and the statistical weight of the configuration in which all phospholipid molecules exist in the liquid-crystal state. Thus, $Z(N)$ and $Q(N)$ differ only on the choice of the reference state and therefore

$$\frac{Z(N)}{Q(N)} = e^{-\Delta G^0/RT} \tag{2}$$

where $\Delta G^0 = G_g - G_f$ is the free energy difference between a bilayer in the all-gel and all-liquid crystalline configurations. The average thermodynamic properties of the system can be derived either from $Z(N)$ or $Q(N)$.

Since the thermodynamic quantities of bilayer systems will be expressed in terms of moles of phospholipid molecules rather than moles of vesicles or bilayers, it is necessary to introduce the 'residue' partition functions $q(N)$ and $z(N)$ defined as,

$$q(N) = [Q(N)]^{1/N};\ z(N) = [Z(N)]^{1/N} \tag{3}$$

In doing this transformation no loss of generality is made since $Q(N)$ and $Z(N)$ can always be recovered by raising $q(N)$ or $z(N)$ to the Nth power.

$q(N)$ and $z(N)$ can be numerically evaluated at any temperature, T, from the average excess enthalpy function, $\langle \Delta h \rangle$. $\langle \Delta h \rangle$ is obtained by direct integration of the excess heat capacity function, $\langle \Delta Cp \rangle$, experimentally obtained from scanning calorimetric data:

$$\langle \Delta h \rangle = \int_{T_0}^{T} \langle \Delta Cp \rangle \, dT = \int_{T_0}^{T} (Cp - Cp,o)\, dT \tag{4}$$

where T_0 is a temperature at which the bilayer is in the all-gel configuration, Cp,o the apperent molar heat capacity of that configuration and Cp the apparent molar heat capacity of the system at any temperature. $q(N)$ and $z(N)$ are given by the following integral relations [12—14]:

$$q(N) = \exp\left(\int_{T_0}^{T} \frac{\langle \Delta h \rangle}{RT^2} \, dT \right) \tag{5a}$$

$$z(N) = \exp\left(\int_{T}^{T_n} (\Delta h - \langle \Delta h \rangle) \frac{1}{RT^2} \, dT \right) \tag{5b}$$

where T_n is a temperature at which the bilayer exists in the all-liquid crystalline configuration, Δh the overall enthalpy change for the transition per mol of phospholipid molecules, and R the universal gas constant. Eqns. 5a and 5b are general and independent of the model or mechanism assumed for the transition [12—14].

Overall thermodynamic parameters. The ratio $z(N)/q(N)$, defined as s, is equal to

$$s \equiv \frac{z(N)}{q(N)} = e^{-\Delta g/RT} \tag{6}$$

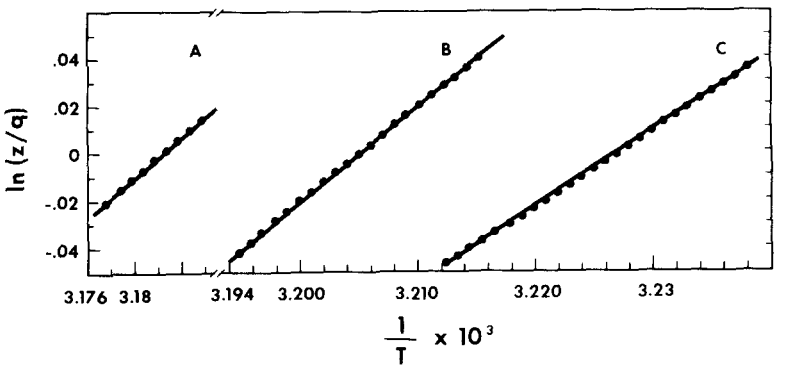

Fig. 1. Van't Hoff representation of $\ln(z/q)$ for the main transition of: (A) multilamellar dipalmitoyl phosphatidylcholine liposomes, (B) multilamellar dipalmitoyl phosphatidylcholine liposomes in equilibrium with 0.047 atm halothane, and (C) sonicated dipalmitoyl phosphatidylcholine vesicles. For these and all calculations throughout this work, the following *Cp* data were used: The data of Mountcastle et al. [3] were used for multilamellar dipalmitoyl phosphatidylcholine liposomes in the absence and in the presence of 0.047 atm halothane. In the case of sonicated single-lamellar dipalmitoyl phosphatidylcholine vesicles, the data of Suurkuusk et al. [2] were used. In this latter case the *Cp* curve was renormalized to 100% vesicle population (Δh = 6.3 kcal/mol of dipalmitoyl phosphatidylcholine) in order to account for the loss of vesicle population due to fusion or aggregation.

where $\Delta g \equiv \Delta G^0/N$ is the Gibbs free energy difference between the gel and liquid crystal states per mol of phospholipid molecules. This relation allows calculation of Δg as a continuous function of temperature without assuming a particular model for the transition.

The van't Hoff representation of $z(N)/q(N)$ is shown in Fig. 1 for the gel-liquid crystalline transition of: (a) multilamellar liposomes prepared from pure dipalmitoyl phosphatidylcholine; (b) multilamellar diplamitoyl phosphatidylcholine liposomes in the presence of 0.047 atm of the gaseous anesthetic halothane; and (c) single-lamellar dipalmitoyl phosphatidylcholine vesicles. The experimental heat capacity profiles have been published elsewhere [2,3]. The residue partition functions $q(N)$ and $z(N)$ were calculated from the experimental C_p data according to Eqns. 4, 5a and 5b. The numerical procedures are given in Refs. 12 and 13. The above experiments were chosen for the purpose of this discussion because they represent two distinct types of perturbations to the system. Addition of the anesthetic predominantly affects the cooperativity and the T_m of the transition, without changing Δh. On the other hand, the thermodynamic characteristics of the gel-liquid crystalline transition in small single-lamellar vesicles differ from those of multilamellar liposomes, both in the degree of cooperativity and the magnitude of Δh.

The results of a linear least-squares analysis of the data in Fig. 1 are summarized in Table I. The agreement between the Δh values obtained in this form and those calculated from the area under the heat capacity curve is excellent. It should be noted that the temperature at which Δg is zero is not necessarily equal to the temperature of the maximum $\langle \Delta Cp \rangle$. This is particularly noticeable for the transition in the presence of halothane. This effect is not due to experimental error in the determination of Δg but merely reflects the fact that the transition is not truly symmetrical. For an assymetrical transition, T_m (defined as the position of the maximum in $\langle \Delta Cp \rangle$) is not equal

TABLE I

THERMODYNAMIC PARAMETERS ASSOCIATED WITH THE MEAN TRANSITION OF DIPALMITOYL PHOPHATIDYLCHOLINE

T_m is the temperature of the maximum in the heat capacity function, $T_{\Delta g=0}$ is the temperature at which $\Delta g = 0$ (from van 't Hoff analysis of $\ln(z/q)$). Δv is calculated from dependence of Δg (see text for details), and taken from references given in brackets. DPPC, dipalmitoyl phosphatidylcholine.

System	T_m	$(T)_{\Delta g=0}$	Δh * (kcal/mol DPPC)	Δh ** (kcal/mol DPPC)	Δs ** (cal/k per mol DPPC)	Δv (l/mol DPPC)	Δv (l/mol DPPC)
Multilamellar liposomes	41.3	41.25	8.6	8.6	27.4	24.5	24.3 [19] 25.7 [18]
Mulitlamellar liposomes plus 0.047 atm halothane	39.5	39.0	8.2	8.1	26.0		
Sonicated single-lamellar vesicles	36.9	36.9	6.3	6.3	20.3		

* Area under the heat capacity curve.
** From van 't Hoff analysis of $\ln(z/q)$.

to the temperature at which $\Delta g = 0$ and, in general, no simple relation exists between the position of the maximum and the value of Δg at that point. This has also proven to be the case for helix-coil transitions of double-stranded nucleic acids [14,16,17].

The capability of the deconvolution analysis for calculating Δg as a function of temperature is particularly important for evaluating other thermodynamic functions like the volume changes accompanying the transition. The volume change, ΔV, per phospholipid molecule is defined as

$$\Delta V = (V_F - V_g) = -\left(\frac{\partial \Delta g}{\partial P}\right)_T \tag{7}$$

and can readily be computed at any temperature from the pressure derivative of Δg. Recently, a novel pressure cell which allows determination of the heat capacity function, at different pressures has been developed and applied to the study of the gel-liquid crystalline transition of dipalmitoyl phosphatidylcholine at several solvent conditions [3]. Thus, one is able to generate a family of Δg curves at different pressures, from which ΔV can be estimated at any temperature without the otherwise necessary assumption that $\Delta g = 0$ at T_m and that Δs is constant. The result of one such analysis for dipalmitoyl phosphatidylcholine multilamellar liposomes using the data of ref. 3, is shown in Table I. The agreement between ΔV values determined by direct density measurements [18—20] and that calculated in the above manner is very good.

Grand partition function. Freire and Biltonen [14] have previously shown that for very large systems ($N \to \infty$), molecular averages and distribution functions can readily be computed from the experimental residue partition functions $q(N)$ and $z(N)$. This method is based on the general result of statistical mechanics that, in the thermodynamic limit, canonical and grand canonical

ensemble averages are identical for all the properties of a system that are bounded functions of N. The grand canonical partition function, $\Xi(\alpha)$, is represented as [14],

$$\Xi(\alpha) = \sum_N e^{-\alpha N} Z(N) = \sum_N e^{-\alpha N} z^N \tag{8}$$

In the thermodynamic limit

$$e^{\alpha} \to e^{\alpha_0} = z \tag{9}$$

Imposing homogeneous boundary conditions and neglecting interactions between clusters, $\Xi(\alpha)$ for the cluster model can be written as a product of cluster grand partition functions Ω_i [14],

$$\Xi(\alpha) = \sum_{\{n_i\}} \prod_i (\Omega_i)^{n_i} \tag{10}$$

where the product runs over all different types of clusters i, and the summation runs over all sets of numbers of clusters $\{n_i\}$ consistent with the specifications of the system. The cluster grand partition functions Ω_i are defined by the equation

$$\Omega_i = \sum_{j=1}^{\infty} e^{-G_{i,j}/RT} e^{-\alpha j} \tag{11}$$

where $G_{i,j}$ is the free energy of a cluster of type i containing j residues. In particular, the gel and liquid crystalline cluster grand partition functions are given by the equations,

$$\Omega_g = \sum_{j=1}^{\infty} s^j e^{-j\alpha} \tag{12a}$$

$$\Omega_f = \sum_{j=1}^{\infty} e^{-j\alpha} \tag{12b}$$

where the liquid crystalline state has been chosen as reference state and s is defined by Eqn. 6.

It should be noted that Eqns. 12a and 12b do not include boundary lipid. Nevertheless, this fact does not imply that boundary or other effects are not accounted for in determining Ω_g and Ω_f. Those effects contribute to the actual value of the activity term, $e^{-\alpha}$, which is contained in the experimental data. Thus, even though one is only able to determine the statistical thermodynamics of phospholipid molecules in the initial gel state and the final liquid crystalline state, this is not meant to imply that the calculations are based on the assumption that gel and liquid crystalline are the only thermodynamic states accessible to a phospholipid molecule.

Average cluster size. In the thermodynamic limit the average size of gel clusters is defined by

$$\langle l_g \rangle = -\left(\frac{\partial \ln \Omega_g}{\partial \alpha}\right)\Big|_{\alpha=\alpha_0} \tag{13a}$$

$$= \frac{\Sigma j s^j e^{-j\alpha_0}}{\Sigma s^i e^{-j\alpha_0}} \tag{13b}$$

but since $s = z/q$ and $e^{\alpha_0} = z$, the average size of gel clusters can be expressed in closed form as

$$\langle l_g \rangle = \frac{q}{q-1} \tag{14}$$

and can be directly determined from the experimental data. For the average size of liquid crystalline clusters, $\langle l_f \rangle$, an analogous procedure leads to $\langle l_f \rangle = z/z - 1$, and can also be determined from the experimental data.

It should be emphasized that the cluster averages $\langle l_f \rangle$ and $\langle l_g \rangle$, are thermodynamically defined quantities and therefore they cannot be immediately identified with their topological counterparts. As defined above, $\langle l_g \rangle$ and $\langle l_f \rangle$ measure the average number of phospholipid molecules in a gel or liquid crystal cluster, which are thermodynamically correlated at any point during the transition.

In Fig. 2 the experimentally obtained averages $\langle l_g \rangle$ and $\langle l_f \rangle$ have been plotted as a function of the temperature. The cluster densities n_g/N and n_f/N are also shown in Fig. 3. These quantities were calculated with the equations:

$$\frac{n_g}{N} = \frac{(1-F_f)}{\langle l_g \rangle}; \quad \frac{n_f}{N} = \frac{F_f}{(\langle l_g \rangle + \langle l_{fg} \rangle)} \tag{15}$$

where n_g and n_f are the number of gel and liquid clusters, respectively. For convenience the boundary lipid will be defined as the interfacial lipid surrounding the liquid clusters. As noted before $\langle l_g \rangle$ and $\langle l_f \rangle$ do not include boundary lipid; however, the relative amount of boundary lipid can be estimated, as will be shown later. F_f is equal to the fraction of phospholipid molecules in the liquid

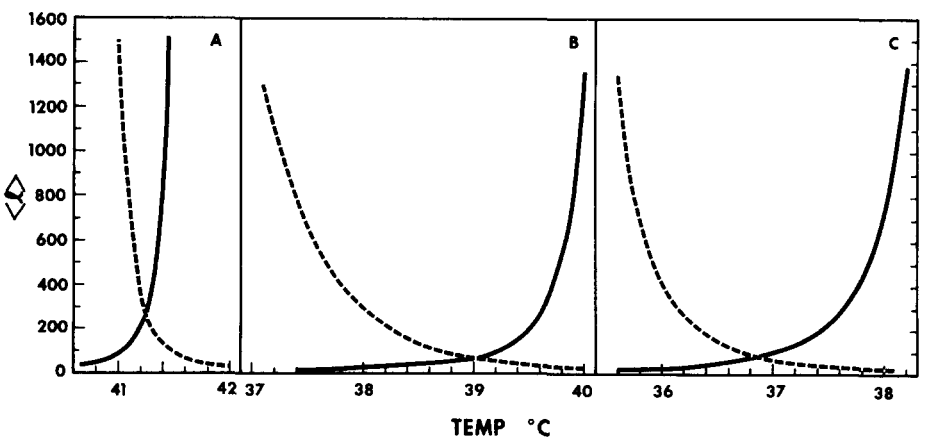

Fig. 2. Average size of gel (- - - - - -) and liquid crystal (———) clusters as a function of temperature for (A) multilamellar dipalmitoyl phosphatidylcholine liposomes, (B) multilamellar dipalmitoyl phosphatidylcholine liposomes in equilibrium with 0.047 atm halothane, and (C) sonicated dipalmitoyl phosphatidylcholine vesicles.

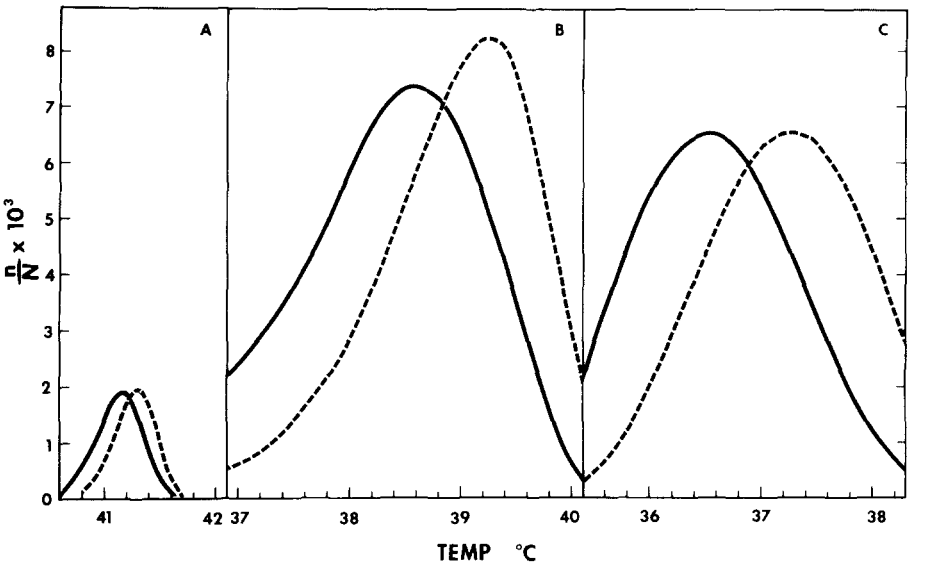

Fig. 3. Gel (- - - - - -) and liquid crystal (———) cluster densities vs. temperature for (A) multilamellar dipalmitoyl phosphatidylcholine liposomes, (B) multilamellar dipalmitoyl phosphatidylcholine liposomes in equilibrium with 0.047 atm halothane, and (C) sonicated dipalmitoyl phosphatidylcholine vesicles.

state and is approximated by

$$F_f = \frac{\langle \Delta h \rangle}{\Delta h} \tag{16}$$

The above calculations are based upon the assumption that, in each case, the system is sufficiently large so that the infinite size approximation is accurate. This assumption appears justified in the case of multilamellar liposomes and, at least, approximate in the case of single lamellar vesicles. Recent theoretical calculations (Freire and Biltonen, in preparation) indicate that size effects are negligible for periodic dipalmitoyl phosphatidylcholine bilayers with more than 10^3 phospholipid molecules.

Several conclusions regarding the transition mechanism of pure dipalmitoyl phosphatidylcholine liposomes, dipalmitoyl phosphatidylcholine plus halothane liposomes and single lamellar dipalmitoyl phosphatidylcholine vesicles can be drawn from these results. First, the cooperativity of the transition is maximal in the case of pure dipalmitoyl phosphatidylcholine multilamellar liposomes. Over the entire transition interval, the average size of gel and liquid clusters is greater in this system. At T_m, the average cluster size is about 300 residues, whereas in the presence of 0.047 atm of halothane or in the case of single lamellar vesicles, it is only 80 residues. This decrease in cluster size is accompanied by an increase in cluster density as shown in Fig. 3. At comparable degrees of melting, there are more clusters per unit area after addition of anesthetic or in the case of small single-lamellar vesicles.

In all cases, the total number of clusters is maximal near the transition midpoint. This increase in the number of clusters at T_m must be accompanied with an increase in the number of gel-liquid neighbors and, therefore, in the amount of lipids at interfacial regions. Incidentally, the enhanced permeability of the

bilayer observed, both at the transition temperature and upon the addition of anesthetics, has been associated to an increase in the total amount of gel-liquid boundaries within the bilayer [9,10]. This quantity can be estimated from the experimental knowledge of $\langle l_f \rangle$.

Fraction of phospholipids at gel-liquid boundaries. Consider the clusters of phospholipids in the liquid state. The average boundary size, $\langle l_{fg} \rangle$, can be expressed as

$$\langle l_{fg} \rangle = \frac{N_{fg}}{n_f} \tag{17}$$

where N_{fg} is the total number of phospholipid molecules in the liquid state in contact with phospholipid molecules in the gel state and where n_f is the total number of liquid clusters. Let us define a fraction, $F_{fg} \equiv N_{fg}/N$, of phospholipids at the gel-liquid boundary; F_{fg} is equal to

$$F_{fg} = \frac{F_f \langle l_{fg} \rangle}{(\langle l_f \rangle + \langle l_{fg} \rangle)} \tag{18}$$

The problem of calculating F_{fg} reduces, then, to evaluate $\langle l_{fg} \rangle$ since $\langle l_f \rangle$ is already known and F_f, the fraction of molecules in the liquid state, can be approximated by $\langle \Delta h \rangle / \Delta h$. This calculation assumes that each phospholipid molecule is either in the gel or liquid crystalline state.

$\langle l_{fg} \rangle$ cannot be estimated unless the dimensionality and the geometry of the liquid clusters are specified. Experimental evidence indicates the absence of significant interactions between monolayers in saturated lecithin bilayers and that their melting procedes independently [21]. These results suggest that phospholipids are only (or predominantly) correlated along the plane of each monolayer, thus giving rise to two-dimensional clusters. Two-dimensional clusters of a given size may form in many different shapes; however, cooperative ineractions will favor those shapes which minimize the number of the energetically less favorable gel-liquid neighbors. Thus, clusters of circular geometry would be the most energetically favorable. This approach is also the most conservative and will yield a lower estimate of F_{fg}.

If we consider $\langle l_{fg} \rangle$ to be a single layer of phospholipids surrounding a circular cluster of $\langle l_f \rangle$ phospholipid molecules, then

$$\langle l_{fg} \rangle = 4(\langle l_f \rangle^{1/2} + 1) \tag{19}$$

The fraction of phospholipids at the gel-liquid boundary, F_{fg}, is then equal to

$$F_{fg} = \frac{4F_f(\langle l_f \rangle^{1/2} + 1)}{(\langle l_f \rangle^{1/2} + 2)^2} \tag{20}$$

and can be estimated from the experimental data. In Fig. 4 we have plotted F_{fg} as a function of temperature for the gel-liquid transition of pure dipalmitoyl phosphatidylcholine liposomes, liposomes of dipalmitoyl phosphatidylcholine in equilibrium with 0.047 atm halothane and small single-lamellar dipalmitoyl phosphatidylcholine vesicles. In all cases F_{fg} passes through a maximum at the transition temperature; however, in the presence of anesthetic or in the case of single-lamellar vesicles, the relative amount of gel-liquid neighbors is considerably greater than in the case of multilamellar dipalmitoyl phosphatidyl-

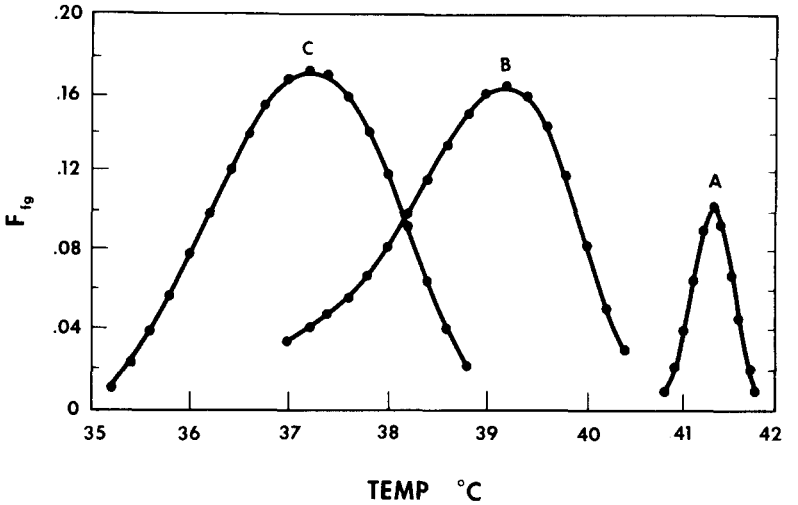

Fig. 4. Fraction of phospholipid molecules at the gel-liquid boundaries as a function of temperature for (A) multilamellar dipalmitoyl phosphatidylcholine liposomes, (B) multilamellar dipalmitoyl phosphatidylcholine liposomes in equilibrium with 0.047 atm halothane, and (C) sonicated dipalmitoyl phosphatidylcholine vesicles.

choline liposomes, over the entire transition region. This is a consequence of the fact that in the two former cases the transition is characterized by a larger number of clusters but which are smaller in size.

Equilibrium fluctuations. The above treatment leads to operational relations which allow calculation of cluster averages from the experimental partition functions q and z. However, these averages do not necessarily provide a very representative picture of the state of the bilayer. A more detailed description will be obtained if, in addition to the cluster averages, the relative fluctuations (standard deviation and dispersion) of these quantities around their mean value are known. This information is also contained in the partition function.

The relative probability, $P_g(l)$, of finding a gel cluster of l phospholipids among all gel clusters is [14]

$$P_g(l) = \frac{s^l \, e^{-\alpha} \, 0^l}{\Omega_g} \tag{21a}$$

$$= \frac{q-1}{q^l} \tag{21b}$$

The moment generating function associated with the probability distribution function, $P_g(l)$, is defined as

$$\langle l_g^k \rangle = \sum_{l=1}^{\infty} l^k \, P_g(l) \tag{22}$$

and can be expressed in terms of the experimental partition function q as

$$\langle l_g^k \rangle = (q-1) \sum_{l=1}^{\infty} l^k \, q^{-l} \tag{23}$$

Eqn. 23 allows us to express all the moments of the distribution in closed form. In particular, the first three moments are

$$\langle l_g^0 \rangle = 1 \tag{24}$$

$$\langle l_g^1 \rangle = \frac{q}{q-1} = \langle l_g \rangle \tag{25}$$

$$\langle l_g^2 \rangle = \frac{q(q+1)}{(q-1)^2} \tag{26}$$

The dispersion of the distribution, Δ^2, defined in terms of the first and second moments, is

$$\Delta^2 = \langle l_g^2 \rangle - \langle l_g \rangle^2 = \langle l_g \rangle (\langle l_g \rangle - 1) \simeq \langle l_g \rangle^2 \tag{27}$$

and indicates a broad distribution with an average fluctuation, Δ, approximately equal to the average cluster size.

In Fig. 5 the function $lP_g(l)/\langle l_g \rangle$ evaluated at $\Delta g = 0$ has been plotted as a function of l for dipalmitoyl phosphatidylcholine pure and dipalmitoyl phosphatidylcholine plus 0.047 atm halothane liposomes. This function is equal to the relative probability that a residue in the gel state is in a cluster of size l. As shown in Fig. 5, the maximum value occurs at $\langle l_g \rangle$, however, the distribution is very broad and the probability of finding phospholipid molecules in clusters of different size than the average is relatively high. This is particularly true for clusters whose size is larger than the average, a consequence of the fact that the third moment of the distribution is positive. The nature of the probability distribution function in this ensemble is such that, at any point during the transition, there are more clusters whose size are smaller than the average and more phospholipid molecules populating clusters whose size are larger than the average. Monte Carlo simulations of cluster distributions in a two-dimensional lattice, like those performed by Tsong et al. [10], qualitatively illustrate this pattern.

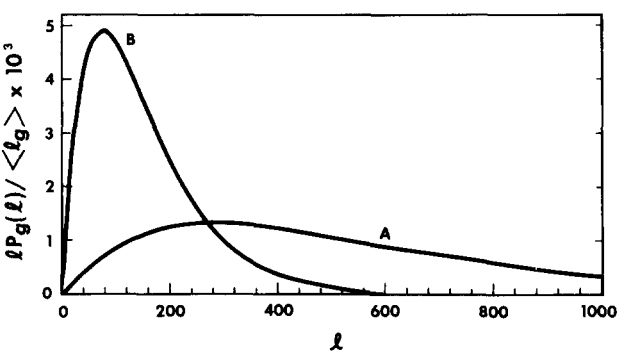

Fig. 5. Relative probability that a phospholipid molecule in the gel state is in a gel cluster of size l at the transition mixpoint. (A) Multilamellar dipalmitoyl phosphatidylcholine liposomes and (B) multilamellar dipalmitoyl phosphatidylcholine liposomes in equilibrium with 0.047 atm halothane.

Discussion

The validity of the theoretical framework and the internal consistency of the deconvolution analysis is demonstrated by the accuracy with which the overall thermodynamic parameters (Δh, Δs and ΔV) of the transition can be calculated from the residue partition functions $q(N)$ and $z(N)$. These calculations are general and require no special assumptions on the system under consideration.

Calculation of cluster distribution functions, on the other hand, requires the fulfillment of the assumption that the cluster model is applicable and that the system is sufficiently large so that $q(N)$ is independent of N. As discussed before, this latter assumption appears justified even in the case of single lamellar vesicles. Thus, the smaller cooperative behavior observed in single lamellar vesicles can be atributed to a reduction in the effective range of interactions between phospholipid molecules rather than to a decrease in the number of phospholipids per bilayer per se. This effect arises from differences in molecular packing dictated by the smaller radius of curvature of the bilayer. This interpretation is consistent with Raman spectroscopic data which indicates that the gel configuration in single lamellar vesicles is more disordered than the gel configuration in multilamellar liposomes [22].

The above results indicate that the gel-liquid transition proceeds through the formation of liquid clusters and that the size of these clusters is affected by external variables such as the presence of anesthetics in the system. Addition of anesthetics makes the formation of gel-liquid crystal neighbors more energetically favorable. Thus, at any degree of melting, the transition is characterized by a larger number of clusters which are smaller in size.

Those phospholipid clusters which exist in the transition region are not static domains but dynamic entities characterized by rather large fluctuations in size. Since the gel-liquid transition is accompanied by a positive volume change, fluctuations in the size of the clusters must be accompanied by localized density and volume fluctuations, as has been pointed out by Mountcastle et al. [3]. Since the magnitude of these fluctuations is approximately equal to the average cluster size, a decrease in the cooperative behavior of the system will be accompanied by an increase in the boundary lipid and a decrease in the magnitude of the local fluctuations.

Recently, boundary lipid and local fluctuations have been associated with the functioning of biological membranes. For example, gel-gel or liquid-liquid neighbors are structurally better matched than gel-liquid neighbors; therefore, it seems plausible that permeation of small molecules through the bilayer would be enhanced by an extra amount of gel-liquid neighbors. This situation occurs near T_m and in the presence of anesthetics. Volume fluctuations, on the other hand, may, in principle, generate relatively large local free volumes through which larger molecular weight substances could penetrate the bilayer. For example, a fluctuation of 300 molecules in the size of a dipalmitoyl phosphatidylchonie cluster is equivalent to a volume fluctuation of approx. 7/ mol, i.e. a volume fluctuation comparable to the size of a small molecular weight protein. The actual incorporation of a protein into the membrane will depend on the absolute probability of occurrence of a fluctuation of a minimum size and the time scale in which these fluctuations occur. The

fluctuations calculated in this work are equilibrium fluctuations and, as such, they correspond in the time domain to an averaging process over an infinite period of time. Nevertheless, it is interesting to note that various protein-lipid related phenomena have been shown to possess an anomalous behavior near T_m [23—25], i.e. where the occurrence of these fluctuations is maximal.

Two different theoretical approaches attempting to quantitatively estimate different cluster averages exist in the literature; that of Marsh and co-workers [9,26], in which the Zimm and Bragg model originally developed for helix-coil transitions is generalized to two dimensions, and that of Tsong et al. [10] using Fisher's approximation to the two-dimensional Ising model. These two models provide the same basic qualitative picture of the transition and are in general agreement with our present results. Computer simulations by Tsong et al. [10] and Marsh et al. [9,26] have clearly shown that the fraction of boundary lipid passes through a maximum at the transition temperature and that this behavior is due to the formation and disruption of clusters. These computer simulations agree with our calculations based upon experimental partition functions. Incidentally, the calculated fractions of boundary lipid are in all three cases of the same order of magnitude (3—10%).

Even though theoretical models play a useful role in describing the qualitative features of the system they are limited by the assumptions and constraints required to evaluate the partition function. In most cases, the lattice structure must be simplified and various molecular forces must be neglected in order to obtain a mathematically tractable model. These difficulties, existing in all model-based calculations, are circumvented by the deconvolution analysis since the model independent partition function is numerically evaluated from the experimental heat capacity function.

Acknowledgements

This work was supported by grants from the National Institutes of Health (GM-20637) and the National Science Foundation (PCM72-23245-A01).

References

1 Mabrey, S. and Sturtevant, J. (1976) Proc. Natl. Acad. Sci. U.S. 73, 3862—3866
2 Suurkuusk, J., Lentz, B., Barenholz, Y., Biltonen, R. and Thompson, T. (1976) Biochemistry 15, 1393—1401
3 Mountcastle, D., Biltonen, R. and Halsey, M. (1978) Proc. Natl. Acad. Sci. U.S., in press
4 Nagle, J. (1973) J. Chem. Phys. 58, 252—264
5 Marcelja, S. (1974) Biochim. Biophys. Acta 367, 165—176
6 Scott, H. (1975) J. Chem. Phys. 62, 1347—1353
7 Jacobs, R., Hudson, B. and Andersen, H. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3993—3997
8 Jackson, M. (1976) Biochemistry 15, 2555—2561
9 Marsh, D., Watts, A. and Knowles, P. (1976) Biochemistry 15, 3570—3578
10 Tsong, T., Greenberg, M. and Kanehisa, M. (1977) Biochemistry 16, 3115—3121
11 Wickner, W. (1977) Biochemistry 16, 254—258
12 Freire, E. (1977) Ph.D. Dissertation, University of Virginia
13 Freire, E. and Biltonen, R. (1978) Biopolymers 17, 463—479
14 Freire, E. and Biltonen, R. (1978) Biopolymers 17, 481—496
15 Lee, A.G. (1977) Biochim. Biophys. Acta 427, 237—281
16 Applequist, J. (1969) J. Chem. Phys. 50, 600—609
17 Blake, R. (1973) Biophys. Chem. 1, 24—34

18 Nagle, J.F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3443—3444
19 Blazyk, J.F., Melchior, D.L. and Stein, J.M. (1975) Anal. Biochem. 68, 586—599
20 Wilkinson, D.A. and Nagle, J.F. (1977) Biophys. J. 17, 48a
21 Sillerud, L.O. and Barnett, R.E. (1978) Biochemistry, in press
22 Mendelsohn, R., Sunder, S. and Bernstein, H.J. (1976) Biochim. Biophys. Acta 419, 563—569
23 Op Den Kamp, J.A.F., Kauerz, M.Th. van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 169—177
24 Wilschut, J.C., Regts, J., Westenberg, H. and Scherphof, G. (1976) Biochim. Biophys. Acta 433, 20—31
25 Linden, C.D., Wright, K.L., McConnell, H.M. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2271—2275
26 Marsh, D., Watts, A. and Knowles, P.F. (1977) Biochim. Biophys. Acta 465, 500—514